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13-Subg
Lipid Nanotubes as a Tool for Studying Nanoscale Proteo-Lipid Domains
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Membrane curvature can play a decisive role in demixing of membrane bound proteins, allowing for formation of fluid-like or gel-like proteo-lipid domains responsible of distinct shape (shape creators) and/or function (e.g. topological remodeling). We take advantage of the nanoconfinement offered by the lipid membrane tethers or lipid nanotubes to access such domains and reveal the fine details of their dynamic life. By combining conductance and fluorescence measurements on a lipid nanotube we are able to monitor the nucleation, stepwise growth and disassembly of individual dynamin1 nanodomains and observe reversible changes in membrane shape and topology produced by them. The sensitivity of this method relies on the nanoconfinement of the tube and the correlative analysis of the data, and gives the spatial and temporal resolution needed for the study of dynamic elasticity of non-homogeneous membranes at nanoscales.

14-Subg
How Cells Exploit Forces to Sense and Respond to their Environments
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Cells recognize physical features in their environments by exploiting mechanical forces generated by their motors which pull on distal extracellular anchoring points. Filopodia have been described previously as the "sticky fingers" that help cells to explore their environments and help immune cells to clear pathogens. Here we will ask how cells exploit the interplay between filopodia and lamellipodia to explore their environments, recognize surface properties and find their pray. Myosin-generated tensile forces acting on filopodia are utilized by cells to pull on external adhesive objects. If the external objects can be deflected, the filopodia adhesion will grow as filopodia and object align, but filopodia often peel off from flat surfaces. Tensile forces acting on filopodia are thus used by cells to distinguish between deflectable nanofibrillar environments and flat surfaces. Only if lamelliopdia are in contact with flat surfaces, the tensile forces acting on filopodia can steer the protrusion of lamellipodia. This synchronized movement is needed for the physical removal of surface adhering pathogens for example by macrophages. Membrane tension might play a still poorly understood role in the local coordination of events.

15-Subg
Determining the In-Plane and Out-of-Plane Structure of Model Membranes; Two Recent Examples
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With the exception of hydrogen, neutrons are found in all atomic nuclei. Importantly, unlike X-rays, neutrons are able to differentiate between the different isotopes of the same element. In biology, the classic example is the isotopic substitution of hydrogen for deuterium, allowing one to selectively tune the sample's contrast *in situ* with minimal or no change to its native structure. Biological membranes are believed to exist in a disordered state, a fact that presents unique challenges to elucidating their fine structure. In the case of model membranes, to overcome this difficulty we have developed the Scattering Density Profile (SDP) model, which combines neutron and x-ray scattering data, with molecular dynamics simulations to yield robust structural data, including the much sought after area per lipid needed by simulators to refine their force fields. In addition to one-dimensional structural data along the membrane, we have recently exploited the contrast variation offered by neutron scattering (exchange of hydrogen for deuterium), to study - with unprecedented accuracy - the lateral phase separation (in-plane structure) of so-called "raft" forming mixtures. We hope that in the near future we will apply this knowledge to address the question that has vexed biologists and confounded experimentalists for over 40 years: do membrane domains exist *in vivo*?

16-Subg
Membrane Fusion by X-Rays: From Model Membranes to Organelles
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Understanding the physical mechanisms underlying membrane fusion requires a multi winged approach, involving model systems as well as biological membranes. We study fusion intermediates occurring in form of ordered passages or stalks connecting neighbouring bilayers in multilamellar model membrane stacks. The stalks exhibit long range crystalline order with rhombohedral symmetry in a fluid 'host' membrane stack, which is studied by high resolution x-ray diffraction under grazing incidence angles. Information on membrane curvature, and hydration interaction can be revealed by analyzing the quantitative electron density maps, collected for controlled environmental parameters and membrane composition [1]. Phase diagrams can be analyzed in view of stabilizing or destabilizing agents for stalk formation.
While in these equilibrium phase, dehydration forces bring bilayers together favoring at some point the formation of stalks, it is specific membrane proteins and their interaction which set the local boundary conditions for membrane apposition in biological membrane fusion. In view of studying fusion in the presence of SNARE proteins, we have started a x-ray structural characterization of synaptic vesicles (SV) by small-angle x-ray scattering, and currently extent this work towards studies of SV dockled to and interaction with model bilayers [2].
Finally we present a novel high resolution x-ray imaging scheme capable of yielding a magnified hologram of a freely suspended lipid membrane illuminated by highly divergent and coherent x-ray beams. We propose this setup to image fusion trajectories at high resolution in future experiments [3].
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17-Subg
Some of my Greatest Mistakes
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2014 Thomas E. Thompson Award

Subgroup: Bioenergetics

18-Subg
FOF1-ATP Synthase Dimers and The Mitochondrial Permeability Transition Pore from Yeast to Mammals
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The mitochondrial permeability transition pore (PTP) is a voltage-dependent channel that allows solutes of molecular mass $\leq$ 1.5 kDa to equilibrate across the inner membrane. Matrix Ca^{2+} accumulation, together with Pi and a set of compounds collectively called "inducers", is necessary to induce PTP opening. In mammals cyclosporin (Cs) A desensitizes the PTP through its binding to cyclophilin D, a matrix protein that facilitates PTP opening. Yeast and Drosophila mitochondria also possess Ca^{2+}-activated channels which, at variance from the mammalian PTP, are insensitive to CsA and inhibited rather than activated by Pi. We show (i) that the permeability properties of the Drosophila channel, which displays selectivity toward Ca^{2+} and H^{+}, are not modified by expression of human cyclophilin D; and (ii) that, in keeping with our recent demonstration that the mammalian PTP forms from dimers of the FOF1-ATP synthase, Ca^{2+}-dependent currents can be elicited in reconstitution experiments with purified dimers of the yeast enzyme. We are currently investigating the effect of genetic ablation of FOF1-ATP synthase subunits that mediate dimerization on PTP opening in yeast, Drosophila and mammalian mitochondria. Our findings suggest that the PTP-forming ability of FOF1-ATP synthase has been conserved in evolution, and that the channels display species-specific features.

19-Subg
The C-Subunit of the ATP Synthase Forms the Pore of the PTP
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Mitochondria maintain tight regulation of inner mitochondrial membrane (IMM) permeability to sustain ATP production. Stressful events cause cell

Ca^{2+} dysregulation followed by rapid loss of IMM potential known as permeability transition (PT), which produces osmotic shifts, metabolic dysfunction and cell death. The molecular identity of the mitochondrial PT pore (mPTP) is still in question. We had described previously that, through protein-protein interaction with the beta subunit of the ATP synthase, the anti-apoptotic protein Bcl-xL decreased an inner membrane leak conductance to increase bioenergetic efficiency during neuronal activity. To identify the source of the leak, we used patch clamp recording of submitochondrial vesicles. We have now shown that the leak channel most likely regulated by interaction with the beta subunit is the c-subunit ring of the F1FO ATP synthase and we further suggest that this channel forms the mPTP. High Ca^{2+} enlarges the c-subunit ring and uncouples it from Ca^{2+}/ cyclosporine A (CsA) binding sites in the F1 of the ATP synthase. Depletion of the c-subunit prevents PT and attenuates cell death, while increasing the expression or conductance of the c-subunit channel sensitizes cells to death. Physical uncoupling of F1 from FO occurs when PT is induced, and an antibody specific to the c-subunit inhibits c-subunit channel conductance and prevents calcium-induced IMM channel activity. We conclude that a highly regulated c-subunit leak channel is the mPTP.

20-Subg
Mitochondrial Uncoupling and Thermogenesis
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No abstract.

21-Subg
New Mitochondrial Potassium Channels
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Mitochondrial potassium channels play an important role in cytoprotection. The following potassium channels have been described in the inner mitochondrial membrane: the ATP-regulated potassium channel, the large conductance calcium activated potassium channel, the voltage-gated potassium channel and the twin-pore domain TASK-3 potassium channel. Potassium channels in the inner mitochondrial membrane are modulated by inhibitors and activators (potassium channel openers) previously described for plasma membrane potassium channels. The majority of mitochondrial potassium channel modulators exhibit a broad spectrum of off-target effects. These include uncoupling properties, inhibition of the respiratory chain and effects on cellular calcium homeostasis. Therefore, the rational application of channel inhibitors or activators is crucial to understanding the cellular consequences of mitochondrial channel inhibition or activation. In this paper, new observations on mitochondrial potassium channel will be discussed: 1). their molecular identity, 2). their interaction with potassium channel openers and inhibitors and 3). their functional role.

22-Subg
Inhibition of a Mitochondrial Potassium Channel as a New Therapeutic Strategy for Chronic Lymphocytic Leukemia
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Ion channels are involved in the regulation of proliferation and apoptosis and are emerging as promising oncological targets. We have recently identified three membrane-permeant inhibitors of the Kv1.3 potassium channel as inducers of apoptosis. These inhibitors induce mitochondrial membrane potential changes, increase of ROS release and opening of the permeability transition pore finally leading to cytochrome c release and cells death. Death occurs only when Kv1.3 is active in the inner mitochondrial membrane (mtKv1.3) and also in the absence of Bax and Bak. Efficiency of one of these inhibitors to reduce tumor volume in vivo was demonstrated in a melanoma orthotopic mouse model (Leanza, et al., 2012 EMBO Molecular Medicine). The same signaling pathway and a selective action of the Kv1.3 inhibitors was observed in B lymphocytes of patients suffering of Chronic lymphocytic leukemia (B-CLL) (Leanza, et al., 2013 Leukemia). Regarding the mechanism of selectivity, we defined that synergy between the level of Kv1.3 expression and an altered redox state in B-CLL cells determines the susceptibility of these cells. Since Kv1.3 inhibitors kill B-CLL by direct interference with mitochondrial functions, they act on these malignant cells independent of classic prognostic factors and Bcl-2 overexpression. To generalize our findings, in addition to melanoma and B-CLL cells, the effect of these inhibitors on cell survival was measured on different cancer cell lines expressing Kv1.3. A correlation between Kv1.3 expression and susceptibility to mtKv1.3 inhibitors was found.

23-Subg
Protein Acylation Regulates Metabolism
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Proteins are decorated with a suite of chemical modifications, which regulate their activity and overall metabolic homeostasis. The most well-studied lysine modification is acetylation, and hyperacetylation of several proteins leads to metabolic dysfunction and potentially contributes to human disease. More recently, new chemical modifications that regulate protein activity have emerged, including succinylation and malonylation, however little is known about the biology regulated by these modifications. The suite of acyl-based chemical modifications of mitochondrial proteins is regulated by a family of NAD^+-dependent deacetylase enzymes call the sirtuins (SIRT1-7), which have also been termed ''deacylases'' for their new enzymatic activities. We recently discovered new protein modifications present on cellular proteins, which provides important insight into the regulatory role of the sirtuins.

24-Subg
Regulation of Mitochondrial Protein Function by PTMS during Acute Andchronic Nutrient Stress
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Mitochondria are complex and dynamic organelles that are essential to the survival of nearly every eukaryotic cell. To generate a foundation for systematic investigations of mitochondrial function and adaptation, we recently established a protein compendium of these organelles across a wide range of tissues from healthy mice. This resource, termed MitoCarta, provides a robust, yet static view of the mitochondrial proteome. We are now applying MitoCarta as a framework for quantifying how mitochondrial proteins and post-translational modifications (PTMs, e.g., phsophorylation and acetylation) change during acute and chronic metabolic perturbations, and to elucidate the role of these changes in regulating mitochondrial activity. To do so, we blend state-of-the art multi-plexed mass spectrometry-based proteomics with focused biochemistry and molecular biology approaches.
In particular, we have recently taken this approach to capture the mitochondrial proteome dynamics during fasting, the onset of obesity, aging, caloric restriction and acute iron deprivation. Our analyses have revealed hundreds of dynamic phosphorylation and acetylation events and have produced quantitative, searchable maps of mitochondrial alterations across a spectrum of metabolic states. We have leveraged these data to demonstrate that key steps in ketogenesis, the TCA cycle, branched-chain amino acid degradation and fatty acid oxidation are regulated by reversible PTMs, and that the mitochondrial oxidative phosphorylation machinery is highly calibrated to cellular iron content. Moving forward, we plan to further elucidate the mitochondrial signaling network by identifying the regulatory enzymes (e.g., kinases, acetyltransferases, etc.) responsible for managing mitochondrial PTMs, and to define the functions of uncharacterized mitochondrial proteins mutated in human disease.

Subgroup: Intrinsically Disordered Proteins

25-Subg
Folding Upon Binding - Is it just a Simple Protein Folding Problem?
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One particularly useful approach for investigating protein folding is 'The Fold Approach', which involves a detailed analysis of the folding of several topologically, structurally and/or evolutionarily related proteins in order to discern patterns and trends in folding (stability, pathways and mechanisms). We are now applying this approach to the folding of intrinsically disordered proteins.
The three systems we are studying all involve formation of helical structure when a disordered protein binds its target. The properties of these systems are vastly different, from μM - nM binding affinity and with association and disassociation rate constants varying by many orders of magnitude. This brings different challenges for determining the kinetics of assembly and disassembly. We can use protein engineering approaches, pioneered in protein folding studies to investigate the mechanism of binding, ask whether the presence of residual structure in the disordered peptide affects binding, and investigate the importance of solvent.
Our results challenge some of the long held views on IDPs - how important residual structure is to binding; the idea that IDPs are special in conferring high specificity combined with low affinity. Can we relate biophysical properties to specific IDP functions?